A New Radio-resistant Yeast of *Trichosporon oryzae* nov. sp. Isolated from Rice†

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A radio-resistant asporogenous yeast has been isolated from unpolished new crop rice. On the cultivation of this species, formation of true mycelium, arthrospores and blastospores were observed, and it could be classified as in one group of genus *Trichosporon*. Its taxonomic characteristics were found to be sufficiently different from all the described species in this genus to warrant its description as a new species and was named as *Trichosporon oryzae* nov. sp.

This species exhibited high resistance to gamma-radiation as same as *Pullularia pullulans*, and the D_{10} value of radio-resistant cells was to be ca. 120 krad in 0.067 M phosphate buffer which was about 4 to 5 times larger than that of *Saccharomyces cerevisiae*. In this study, high resistance to gamma-radiation was also observed on the species of *Trichosporon capitatum* and *Tr. inkin*.

In the previous studies^{1~3)} on the effect of gamma-radiation on the microflora of rice, we had isolated two species of radio-resistant microorganisms such as the so called "red *Pseudomonas*" and the radio-resistant asporogenous yeast. The radio-resistant *Pseudomonas* is a characteristic organisms of normal unpolished new crop rice, and was named as *Pseudomonas radiora* nov. sp. in a previous paper.⁴⁾ The radio-resistant asporogenous yeast was isolated from unpolished new crop rice harvested in Japan when irradiated with a dose of 400 krad or more, and was more radio-resistant than *Ps. radiora* on rice.

In this paper we will describe an experimental study on taxonomy and radiosensitivity, and will make the description of taxonomical characteristics as a new species in the genus *Trichosporon*.

MATERIALS AND METHODS

Determination of taxonomic characteristics. Two strains, No. R1 and R3, tested in this study were isolated from samples of unpolished new crop rice of named "Nipponbare" species produced in Tochigi prefecture

and unknown origin produced in Gunma prefecture in 1971, by using MYG agar containing chloramphenicol (Malt ex. 10 g, yeast ex. 4 g, glucose 4 g, chloramphenicol 10 mg, agar 20 g per liter). The taxonomic study of these strains was carried out mainly by the method of Iizuka and Goto,⁵⁾ and identification was also done according to the manual of J. Lodder *et al.*⁶⁾ Seven strains of the genus *Trichosporon* which were obtained from Centraalbureau voor Schimmelcultures in Netherlands were used for the comparison in this study. These strains were as follows; *Tr. cutaneum* CBS 2466, *Tr. inkin* CBS 5585, *Tr. pullulans* CBS 2532, *Tr. capitatum* CBS 2364, *Tr. fermentans* CBS 2529, *Tr. penicillatum* CBS 5586, *Tr. aculeatum* CBS 5578. In addition, seventeen strains of radio-resistant asporogenous yeast which had isolated from irradiated unpolished rice as reported in the previous papers^{1~3)} were also investigated for the comparison in this taxonomic study.

Radiation sensitivity. Seven strains of the genus *Trichosporon*, *Geotricum* sp. ml-13, *Candida* sp. v3-1, *Saccharomyces cerevisiae* 52a, and the newly isolated strains of R1 and R3 were grown on slants of MYG agar for 7 days. The strains of both *Geotricum* sp. ml-13 and *Candida* sp. v3-1 were isolated from Wiener sausages.⁷⁾ Cells of these strains were suspended and dispersed sufficiently in 0.067 M phosphate buffer solution. After filtration by a No. 3 glass filter, each suspension of cells was irradiated in equilibrium with atmospheric air at about 20°C. Viable cells were determined by counting visible colonies developed after incubation for 3 to 5 days on the MYG agar plate at

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20 to 30°C.

In order to obtain the survival curves, the newly isolated strain of R1 was grown in malt extract medium for 18 hr or 72 hr at 30°C with shaking. After harvest of cells, they were washed twice with 0.067 M phosphate buffer, and were resuspended in the same buffer. These suspensions were irradiated under the bubbling with air through a capillary tube at about 20°C. The irradiation was also carried out in equilibrium with atmospheric air. The gamma-ray source used was the cobalt-60 irradiator of this Institute, and the dose rate at the main irradiation position was 200 krad/hr as determined by a Fricke dosimeter.

RESULTS AND DISCUSSION

Taxonomic characteristics and the description

This species was isolated abundantly from irradiated unpolished new crop rice as reported in previous papers.¹⁻³⁾ There are no difference on taxonomic characteristics between two isolates of No. R1 and R3, and were also identical to those of all other strains isolated from irradiated unpolished rice. This species does not produce ascospores and ballistospores, and sexual reproduction has not been observed. However, formation of true mycelium and pseudomycelium are present, and formation of arthrospores (Fig. 1) and blastospores

FIG. 1. True Mycelium and Arthrospores of *Trichosporon oryzae* nov. sp. No. R1 on Potato-glucose agar, Incubated for 7 days at 30°C. ($\times 400$)

(Fig. 2) are also observed. Budding cells are always formed abundantly (Fig. 3). For these reasons, this species should be classified as a species in the genus *Trichosporon*. From the manual of the classification of the *Trichosporon*

FIG. 2. Blastospores of *Trichosporon oryzae* nov. sp. No. R1 on Potato-glucose agar, Incubated for 7 days at 30°C. ($\times 400$).

FIG. 3. Budding Cells of *Trichosporon oryzae* nov. sp. No. R1 Incubated after 3 days at 25°C in Peptone-yeast ex. -malt ex. water. ($\times 800$).

poron in "The Yeasts,"⁶⁾ this species could be included in the Group I which contains *Tr. cutaneum*, *Tr. inkin* and *Tr. pullulans*. All strains in the Group I always assimilate glucose and lactose oxidatively, and produce extracellular starch-like compounds, and hydrolyze urea. The nutritional study of this newly isolated strains showed the ability to assimilate a large number of carbon compounds as same as glucose and fructose. It can also assimilate potassium nitrate and sodium nitrate abundantly, and hydrolyzes urea.

The nutritional characteristics discussed above strongly suggest that this species resembles to the description of *Trichosporon pullulans*

(Lindner) Diddens et Lodder. However the size of budding cells of this species is very small compared with *Tr. pullulans* and other species. Its optimum temperature for growth is at 27 to 33°C, and is different from that of *Tr. pullulans* which can not grow at 27°C or more. The ability of producing extracellular polysaccharide, which is not starch-like compounds, was also observed in the medium by this species. Compared with other species of *Trichosporon*, it has osmotorelant characteristics and can grow on 50% (w/w) glucose-yeast extract agar. The streak culture of this species shows pale grayish brown to orange-yellow surface, and it could be easily distinguishable from the other species of *Trichosporon* (Fig. 4). For these reasons, this

FIG. 4. Colony of *Trichosporon oryzae* nov. sp. No. R1 on Potato-glucose Agar, Incubated for 28 days at 25°C.

species should be classified as a new species in the genus *Trichosporon*, and is named as *Trichosporon oryzae*. This species exhibits high resistance to gamma-radiation compared with *Tr. cutaneum* or *Tr. pullulans*. It is a characteristic organism of normal unpolished new crop rice, and the main microflora surviving after irradiation with more than 500 krad may comprise this species and *Pseudomonas radiora* as reported in previous papers.¹⁻³⁾ The detailed characteristics of this new species of genus *Trichosporon* is as follows.

Description of Trichosporon oryzae Ito, Iizuka et Sato sp. nov.

Latin Diagnosis

Cultura in aqua peptono et extracto fermenti et maltato continti: ad 25°C, post 3 dies cellulae sunt ovoideae ad elongatae $2\sim3\times2\sim10$, singulae vel binae; mycelium verum et arthrospora perraro formantur; pellicula repens formatur plerumque.

Cultura in striis in agar extracto fermenti et maltato continti: post 25 dies, ad 25°C color subgriseo-fuscus vel aurantio-fluvus, pagina corrugata vel rugosa, velutina vel coriacea, applanata vel elevata, margo integer vel undulatum fimbriatum cum mycelio.

Cultura in lamina vitrea: mycelium verum et arthrospora praesentes; pseudomycelium atque blastospora praesentes.

Fermentatio: nulla.

Assimilato glucosum, galactosum, sucrosus, maltosum, lactosum, *l*-sorbosum, cellobiosum, trehalosum, melibiosum, inulinum, *d*-arabinosum, *d*-ribosum, *l*-rhamnosum, *d*-mannitolum, salicinum, amidum, *d*-xylosum, raffinose, inositolum, acidum succinicum et acidum citricum (varium). Non assimilato ethanolum.

Kalii nitras assimilatur, arbutinum finditur, amyllum formatur, ureum finditur. Vitamina addita non necessaria sunt. Summa temperatura acutus: 27~32°C.

Typus: R1, e *oryza impolitia japonica*, Tokyo, Japonia, leg. H. Ito and H. Iizuka, 8, ii, 1972. Culturae hujus speciei conservantur in "Centraalbureau voor Schimmelcultures," Baarn, the Netherlands.

Growth in peptone-yeast extract-malt extract water: After 3 days at 25°C, budding cells may be abundantly formed, and true hyphae and arthrospores are very scarce. The budding cells are ovoid to elongate, usually single or in pairs measuring $(2\sim3)\times(2\sim10)\mu$. Creeping pellicle is usually formed, in some cultures there are only islets and a ring that may be incomplete.

Growth on yeast extract-malt extract agar: After 25 days at 25°C, the streak culture shows

pale grayish brown to orange-yellow surface, wrinkled to rugose, velvety or coremial, flat or raised, with an entire or undulating border fringed with mycelium.

Glass slide cultures on potato-glucose agar: True mycelium and arthrospores are abundant; pseudomycelium and oval blastospores are also present.

Formation of asexual spores: Absent.

Fermentation: Negative.

Assimilation of carbon compounds: Glucose+, Galactose+, Sucrose+, Maltose+, Lactose+, L-Sorbose+, Cellobiose+, Trehalose+, Melibiose+, Inulin+, D-Arabinose+, D-Ribose+, L-Rhamnose+, D-Mannitol+, Salicin+, Soluble starch+, D-Xylose+, Raffinose+, Inositol+, Succinic acid+, Citric acid±, Ethanol—.

Assimilation of nitrogen compounds: Potassium nitrate+, Sodium nitrate+.

Growth in vitamine-free medium: Positive.

Vitamines stimulating growth: None.

Growth on 50% (w/w) glucose-yeast extract medium: Positive.

Decomposition of arbutin: Positive.

Hydrolysis of urea: Positive.

Formation of polysaccharaide: Positive.

Optimum temperature of growth: 27~32°C.

Source: Japanese unpolished new crop rice (1972).

The taxonomic characteristic of No. R3 was same as No. R1.

Radiosensitivity

The radiosensitivity of each strain of the genus *Trichosporon* and *Trichosporon oryzae* nov. sp. incubated on slant cultures for 7 days was compared with other yeasts under the identical condition of irradiation with doses at 125, 500 and 1000 krad, as shown in Table I. As is evident from the table, the radio-resistances of *Tr. capitatum*, *Tr. inkin* and *Tr. oryzae* were much higher than that of the other species of *Trichosporon*, while those of *Tr. pullulans* and *Tr. cutaneum* were not so high as same as *Saccharomyces cerevisiae* and *Candida* sp. v3-1.

In order to obtain the survival curve of *Tr. oryzae*, cells were harvested and irradiated as described in the METHODS. After 18 hr incubation in Malt extract medium, all of the population were observed as to be vegetative cells under a microscope, and a biphasic dose-survival curve was obtained as shown in Fig. 5 when irradiated under the condition of air-bubbling. In the case of 3 days incubation under the condition of vigorous shaking, all of the population were composed by blastospores, and its survival curve was mainly composed by single-hit exponential curve as shown by dotted line in Fig. 5. These results suggests that the dormant cells of blastospore are more sensitive to gamma radiation than vegetative cells under the identical condition of exposure.

As is evident from Table II, the radiosensitivity of this new species *Tr. oryzae* is

TABLE I. RADIATION SENSITIVITIES OF TYPICAL STRAINS OF GENUS *Trichosporon* INCUBATED ON MYG AGAR SLANTS FOR 7 DAYS

Strain	125 krad	Survival fraction 500 krad	1000 krad
<i>Tr. oryzae</i> nov. sp. R1	3.8×10^{-2}	4.4×10^{-3}	8.5×10^{-4}
<i>Tr. oryzae</i> nov. sp. R3	4.5×10^{-2}	2.7×10^{-2}	1.4×10^{-3}
<i>Tr. cutaneum</i> CBS2466	3.0×10^{-2}	3.7×10^{-6}	—
<i>Tr. inkin</i> CBS5585	1.7×10^{-1}	5.1×10^{-3}	1.7×10^{-3}
<i>Tr. pullulans</i> CBS2532	6.9×10^{-2}	2.2×10^{-5}	—
<i>Tr. capitatum</i> CBS2364	9.5×10^{-1}	4.3×10^{-1}	3.0×10^{-2}
<i>Tr. fermentans</i> CBS2529	1.3×10^{-1}	1.7×10^{-3}	9.8×10^{-5}
<i>Tr. penicillatum</i> CBS5586	2.4×10^{-1}	2.2×10^{-3}	2.2×10^{-5}
<i>Tr. aculeatum</i> CBS5578	3.5×10^{-4}	—	—
<i>Geotricum</i> sp. ml-13	6.9×10^{-1}	1.1×10^{-1}	1.2×10^{-4}
<i>Sacch. cerevisiae</i> 52a	2.8×10^{-2}	1.9×10^{-4}	—
<i>Candida</i> sp. v3-1	1.7×10^{-2}	7.5×10^{-6}	—

TABLE II. RADIATION-SENSITIVITY OF *Trichosporon oryzae* nov. sp. AND OTHER ORGANISMS IN 0.067 M PHOSPHATE BUFFER

Strain	Morphology	Condition	Induction dose (krad)	D ₁₀ value (krad)
<i>Tr. oryzae</i> nov. sp. R1	radio-resistant cells	air-bubbling	250~300	120
"	"	air-equilibrium	300~350	160
<i>Sacch. cerevisiae</i> 52a	vegetative cells	air-bubbling	32	36
<i>Candida</i> sp. v3-1	"	air-bubbling	0	32
<i>Pull. pullulans</i> ⁹⁾	radio-resistant cells	air-equilibrium	320	160

FIG. 5. Survival Curves of *Trichosporon oryzae* nov. sp. No. R1 in 0.067 M Phosphate Buffer Irradiated under the Condition of Air-bubbling.

○—○ vegetative cells harvested after 18 hr of growth in Malt extract medium; ●---● 3 days incubated cells in Malt extract medium.

approximately the same as that of *Pullularia pullulans* (*Aureobasidium pullulans*) as reported by Skou⁸⁾ and Okazawa.⁹⁾ The D₁₀ value of radio-resistant cells of *Tr. oryzae* No. R1 obtained from Fig. 5 was about 4 to 5 times larger for gamma radiation as compared with *Saccharomyces cerevisiae* No. 52a or *Candida* sp. No. v3-1 under the identical condition of exposure. When irradiated under the bubbling with air vigorously, the dose of D₁₀ value of radio-resistant cells of *Tr. oryzae* No. R1 was 120 krad, and its induction dose may be ca. 300 to 400 krad. The radio-resistant cells of *Tr. oryzae* nov. sp. was also found as radio-

resistant as bacterial spores, e.g. of *Bacillus pumilus*, but not heat-resistant as those endospores. In the case of *Pullularia pullulans*, as reported by Okazawa,⁹⁾ its blastospores and clamydospores had a remarkable radiation resistance more than other organs. In this study, vegetative cells of *Tr. oryzae* seems to have a remarkable radio-resistance more than blastospores or arthrospores, and for these reasons it can be presumed that the radio-resistant cells of *Tr. oryzae* nov. sp. may be almost corresponds to the vegetative cells. As reported by Beam,¹⁰⁾ the survival curve of haploid yeast from active culture typically takes a biphasic form, and dividing yeast cells showed more resistant to x-irradiation than interdivisional cells. For these reasons, these radio-sensitivities and the mechanism responsible for such a remarkable radiation resistance must be to investigate still further.

In this study, all other strains of *Tr. oryzae* nov. sp. isolated from irradiated rice also had a same radio-resistivity with strains of No. R1 and R3 in the same condition of exposure, and these species of radio-resistant yeast will be distributed widely in nature.

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